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Responses of rice paddy micro-food webs to elevated CO₂ are modulated by nitrogen fertilization and crop cultivars

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6 **Responses of rice paddy micro-food webs to elevated CO₂ are modulated by nitrogen**
7 **fertilization and crop cultivars**

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Abstract

Elevated atmospheric CO₂ concentrations (eCO₂) often increase plant growth but simultaneously lead to the nitrogen (N) limitation in soil. The corresponding mitigation strategy such as supplementing N fertilizer and growing high-yielding cultivars at eCO₂ would further modify soil ecosystem structure and function. Little attention has, however, been directed toward assessing the responses of soil food web. We report results from a long-term free air CO₂ enrichment (FACE) experiment in a rice paddy agroecosystem that examined the responses of soil micro-food webs to eCO₂ and exogenous nitrogen fertilization (eN) in the rhizosphere of two rice cultivars with distinctly weak and strong responses to eCO₂. Soil micro-food web parameters, including microfauna (protists and nematodes) and soil microbes (bacteria and fungi from phospholipid fatty acid (PLFA) analysis), as well as soil C and N variables, were determined at the heading and ripening stages of rice. Results showed that eCO₂ effects on soil micro-food webs depended strongly on N fertilization, rice cultivar and growth stage. eCO₂ stimulated the fungal energy channel at the ripening stage, as evidenced by increases in fungal biomass (32%), fungi:bacteria ratio (18%) and the abundance of fungivorous nematodes (64%), mainly due to an enhanced carbon input. The eN fueled the bacterial energy channel by increasing the abundance of flagellates and bacterivorous nematodes, likely through alleviating the N-limitation of plants and rhizosphere under eCO₂. While eCO₂ decreased the abundance of herbivorous nematodes under the weak-responsive cultivar by 59% and 47% with eN at the heading and ripening stage, respectively, the numbers of herbivorous nematodes almost tripled ($\times 2.9$; heading) and doubled ($\times 1.6$; ripening) under the strong-responsive cultivar with eCO₂ at eN due to higher

root quantity and quality. Structural equation model (SEM) showed that lower trophic-level organisms were affected by bottom-up forces of altered soil resources induced by eCO₂ and eN, and effects on higher trophic level organisms were driven by bottom-up cascades with 69% of the variation being explained. Taken together, strategies to adapt climate change by growing high-yielding crop cultivars under eCO₂ may face a trade-off by negative soil feedbacks through the accumulation of root-feeding crop pest species.

Key-words: Global change; Crop cultivar; Rhizosphere; Soil food webs; Root microbiome; Soil fauna

1. Introduction

Increasing evidence indicates that soil biota can modify ecosystem functions in response to climate change (Bardgett & van der Putten, 2014; Garcia-Palacios et al., 2015). The rising atmospheric CO₂ concentration (eCO₂) often increases plant photosynthetic rate and enhances carbon allocation belowground by promoting root exudation and root turnover (Ainsworth & Long, 2005; Hu et al., 1999; Zak et al., 2000). Also, eCO₂ enhances plant water use efficiency through reducing plant stomatal conductance and density (Jackson et al., 1994). Alterations in C supply and water resources can lead to changes in the structure and activities of the soil microbial community and the soil food web (Blankinship et al., 2011; Li et al., 2009; Yeates et al., 2009; Drigo et al., 2008; Drigo et al., 2010). These alterations in soil biota and their interactions may in return affect plant productivity through modifying plant-microbe interactions and nutrient availability (Bardgett & van der Putten, 2014), highlighting the need to adopt appropriate management practices in agroecosystems for adaption to future climate change.

Some crop cultivars have been found to respond with increased yield to eCO₂, and breeding such ‘positively responsive’ cultivars recently received major attention as a strategy to optimize crop production under future climate regimes (Brummer et al., 2011; Ziska et al., 2012; Korres et al., 2016). Yield of rice is highly responsive to eCO₂ (Liu et al., 2008a; Shimono & Bunce, 2009) with indica varieties being more responsive to eCO₂ than japonica varieties (Hasegawa et al., 2013; Zhu et al., 2015). Therefore, active selection and breeding for CO₂ responsiveness among rice varieties was suggested as an effective strategy to increase global yields and maintain food security under future global climate change scenarios (Ziska

et al., 2012). Meanwhile, to satisfy the nutrient demand of high-yielding cultivars, more N fertilizer is needed (applied as exogenous N or eN). While low N availability in natural ecosystems may limit plant responses to eCO₂, high N inputs can mitigate N limitation on crop growth in agroecosystems (Reich et al., 2006; Feng et al., 2015). N fertilization may also alleviate N constraints on soil microbes under eCO₂ (Hu et al., 2001; Luo et al., 2004). Yet, the net-effect of different responsive cultivars to eCO₂ in N-enriched soils on soil food web and potential feedbacks to plant production have rarely been examined (van der Putten et al., 2016).

The structure complexity of the soil food web, as an integrated indicator of soil biological interactions that drive soil functioning, can significantly affect crop production in two major aspects (Bardgett & Wardle, 2010; Neher, 2010). First, soil microbes and soil food web interactions mediate nutrient cycling. For example, bacterivorous and fungivorous (i.e. microbivorous) protists and nematodes directly affect the turnover of microbial biomass and the availability of plant nutrients (Bardgett & Wardle, 2010; Eisenhauer et al., 2012). The early syntheses found that these microbivores contributed almost 30% of N mineralization in agroecosystem (Griffiths, 1994; Trap et al., 2016), indicating the pivotal significance of microbial grazers in N cycling. Similar to nematodes, protists have diverse feeding strategies including bacterivores, fungivores, and omnivores (Geisen et al., 2016). Previous studies in grasslands and forests have found eCO₂ tended to favor soil fungi while eN stimulated bacterial growth (Drigo et al., 2008; Garcia-Palacios et al., 2015). Second, root parasites among soil microfauna, such as herbivorous nematodes, can directly affect plant growth (Neher, 2010). Due to this direct trophic link, changes to roots in response to eCO₂ and eN

may affect root herbivore performance by altering the quality and quantity of food resources (Robinson et al., 2012). eCO₂ often leads to a higher C:N ratio in plant tissue, and studies of aboveground insects found that herbivores must compensate for the differences in elemental ratios between the food and their requirements through over-grazing (Molles, 2013). However, it is unclear what factors and/or processes will drive the structure and functioning of belowground systems in rice paddy soils under future climate change scenarios (Okada et al., 2014).

Taking advantage of the long-term free-air CO₂ enrichment (FACE) platform to assess the responses of different rice cultivars to CO₂ concentration and N fertilization (Zhu et al., 2016), we examined the responses of soil micro-food webs (bacteria, fungi, protists and nematodes) at different growth stages of two rice cultivars with contrasting performance under eCO₂. We hypothesized that (1) the bottom-up control plays a primary role in mediating responses and feedbacks of both the decomposer and herbivorous food webs to eCO₂ and eN, and (2) the responsiveness of rice cultivars to eCO₂ and eN is a major factor affecting food web structure via changes in resource input to soil from plant root.

2. Materials and methods

2.1. Study site and experimental design

An experimental platform of free-air CO₂ enrichment (FACE) was established in 2004, with a rice-wheat rotation system in Zongcun Village (119°42'0" E, 32°35'5" N), Yangzhou City, Jiangsu Province. From 2010, the rice-wheat rotation system was changed to a rice-fallow system. The region has a north subtropical monsoon climate with a mean annual temperature

of 16 °C, and mean annual precipitation of 900-1000 mm. The soil at the study site is a Shajiang-Aquic Cambisol, with 18.4 g·kg⁻¹ total C, 1.5 g·kg⁻¹ total N, 57.8% sand, 28.5% silt and 13.7% clay at 0-15 cm depth.

The experiment had a split-plot design with CO₂ as the main factor, and nitrogen fertilization and rice cultivar as the split plot factors. More details about the FACE system were described by Zhu et al., (2016). In brief, a randomized complete block design was established with two levels of target atmospheric CO₂ concentrations. The atmospheric CO₂ of each FACE ring was enriched by 200 μmol CO₂ mol⁻¹ over the ambient (Fig. 1). It consisted of three replicate rings for the eCO₂ and three for the ambient (hereinafter referred to as aCO₂). All eCO₂ rings were 12.5 m in diameter, with an area of 80 m² that was sampled after rows on the edge were excluded.

We studied treatments with no fertilization (aN) and elevated N fertilization (eN), the latter receiving urea and compound chemical fertilizer (N: P₂O₅: K₂O = 15:15:15, %) at 22.5 g N m⁻² yr⁻¹. Urea was applied as a basal dressing (40% of the total dose) one day prior to rice transplanting, as a top dressing at early tillering (30%) and at the panicle initiation stage (30%). Phosphorous (P) and potassium (K) were applied as a compound fertilizer at 9 g P₂O₅ m⁻² and 9 g K₂O m⁻² one day before transplanting. Two contrasting rice cultivars were planted in aN and eN plots as a split-split-plot in both FACE and ambient rings. Since 2012, an indica rice IYYou084 and a japonica rice WuYunJing, showing strong (+ 30% yield increase) and weak (+ 13% yield increase) responses to CO₂ elevation respectively were planted (Zhu et al., 2015). Compared to the japonica rice, the indica rice was characterized as an increase in net photosynthetic assimilation, root growth and N uptake capability under eCO₂ (Hasegawa

et al., 2013; Zhu et al., 2015) The seedlings were grown under ambient air and were transplanted by hand into the aCO₂ and eCO₂ plots at a density of three seedlings per hill and 24 hills per m² for all six rings on 21st June, 2014.

2.2. Soil sampling and analysis

Rhizosphere soil samples were collected with a 3.5 cm diameter corer (0-15 cm depth) no more than 3.5 cm distant of rice plants (Fig. S1), at rice heading (10th Sep) and ripening (27th Oct) stage in 2014. Before sampling, the field was drained for 5 days to facilitate sample collection. Five soil cores were randomly collected from each treatment in each plot and were combined to form one composite sample per treatment per plot. Soil samples were stored at 4 °C and analyzed within 7 days after sampling.

Dissolved organic carbon (DOC) and nitrogen (DON) was exacted from 10 g fresh soil using 50 mL ultrapure water by centrifugation (8000 rpm, 10 min). The filtrate that passed through a 0.45 mm filter membrane was analyzed with a total C analyzer (Elementar, Germany) and a continuous flow analyzer (Skalar, Holland), respectively. The NH₄⁺-N and NO₃⁻-N were extracted with 2 M KCl in a 1:5 (soil: water) suspension and the suspension was filtered through ashless filter paper. The filtrates were determined by a continuous flow analyzer (Skalar, Holland). Mineral N (MN) was calculated by the sum of NH₄⁺-N and NO₃⁻-N and total extractable nitrogen (Ext N) was calculated by the sum of DON, NH₄⁺-N and NO₃⁻-N. Concerning root sampling, representative samples of three individual rice hills were dug out and pooled from each plot. Roots were carefully washed from the soil, then oven-dried and weighed. The C and N contents of roots were analyzed with an elemental analyzer (Elemental, Germany).

The soil microbial community was characterized using phospholipid fatty acid (PLFA) analysis as described by Blight & Dyer (1959) with slight modifications. GC conditions and nomenclature were as described by Buyer & Sasser (2012). Briefly, 8.0 g freeze-dried soil was extracted with a chloroform-methanol-citrated buffer mixture (25 mL at a 1:2:0.8 volume bases). Lipid classes were separated into phospholipid, neutral and glycolipid by solid phase extraction (SPE) tubes (ANPEL Laboratory Technologies Inc., China) containing 0.5 g anhydrous sodium sulfate. The phospholipids were trans-esterified by a mild alkaline methanolysis (Bossio et al., 1998) and the resulting fatty acid methyl esters were extracted in hexane and dried under N₂. Samples were re-dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE). The fatty acids i14:0, i15:0, a15:0, i16:0, 16:1 ω 7c, i17:0, a17:0, 17:0cy, 18:1 ω 9, 18:1 ω 7c and 19:0cy were chosen as bacterial markers, and 16:1 ω 5c and 18:2 ω 6.9c were used as fungal markers (Ruess & Chamberlain, 2010). Selected PLFAs biomarker associated with specific microbial group see Table S1.

Protists (amoebae and flagellates) were enumerated using a modified most-probable number method (Darbyshire et al., 1974). Briefly, 3.0 g fresh soil was suspended in 30 mL sterile Neff's modified amoebae saline (NMAS) (Page, 1976) and gently shaken (180 rpm) for 30 min on a vertical shaker. Threefold dilution series with tryptic soy broth (TSB) and NMAS at 1:9 v/v were prepared in 96-well microtiter plates in quadruplicates. The microtiter plates were incubated at 15 °C in darkness, and the wells were inspected for presence of protists using an inverted microscope at $\times 100$ to $\times 400$ magnification after 7, 14 and 21 days. Abundance of protists was expressed as the number of individuals per gram of dry soil.

Nematode populations were extracted from 100 g fresh soil using a sequential extraction method (Liu et al. 2008b). After the total numbers of nematodes were counted, 100 specimens per sample were randomly selected and identified to the genus level. If the total number was less than 100, all nematodes were identified. The nematodes were assigned to the following trophic guilds: bacterivore, fungivore, herbivore and omnivore-carnivore (Yeates et al., 1993).

2.3. Statistical analyses

Analysis of Variance (ANOVA) and Fisher's LSD posthoc tests were performed using Statistica 7.1 (StatSoft Inc., Tulsa, OK, USA). Since the measurements were repeated on the same plot over time, repeated measures ANOVA was used to test the effects of CO₂ concentration (ambient and elevated), exogenous N (ambient and elevated), or rice cultivar (weak- and strong-responsive) on soil nutrients, soil microbial biomass and the abundance of soil microfauna across rice growth stages. PLFA profiles of microbial groups data on the nematode communities were analysed by principal component analysis (PCA) using the software package CANOCO 5.0 (ter Braak and Smilauer, Wageningen-UR, The Netherlands).

Structural equation models (SEM) were calculated to investigate how elevated CO₂ and N input impacted soil micro-food webs and bacterial and fungal energy channels in soil (as indicated by PLFA profiles and the trophic structure of soil nematode communities). The SEM were separately calculated for the heading and ripening stage. Root parameters could only be collected at the ripening stage after destructive sampling of rice plants, therefore we presented only the full model for the ripening stage (see Fig. S6 for the model for the heading stage that lacks root parameters).

The *a priori* model evaluated relationships among root C/N, root N, soil environment

(DOC, Ext N, MN), bacteria, fungi, flagellates, amoebae and nematode communities at trophic group level. The SEM was performed in Amos version 17.0.2 (Amos Development Corporation, Chicago, IL, USA) using maximum likelihood estimation procedures. Model fit was assessed by χ^2 -text, the comparative fit index (CFI) and the root square mean error of approximation (RSMEA).

3. Results

3.1. Soil resources and environment

Regardless of rice cultivar and N dose the eCO₂ significantly ($p < 0.05$) increased DOC content by 35% and 38% at heading and ripening stage respectively (Fig. 2a). eCO₂ increased DON by 83% across all treatments at the heading stage, but at the ripening stage, DON was reduced by 38% with eCO₂ only under the strong-responsive rice (Fig. 2b). Also, mineral N was reduced by 27% with eCO₂ at the ripening stage (Fig. 2c), while N fertilization increased mineral N on average by 41%, irrespective of CO₂ and rice cultivar (Fig. 2c).

3.2. Soil microbial community

PCA of the microbial communities (PLFA) showed that eCO₂ tended to amplify the difference imposed by N and cultivar at the heading stage. Microbial communities under the strong-responsive rice were strongly influenced by CO₂ and N (PC1 = 83.5% and PC2 = 8.84%; Fig. S3a). At the heading stage, eCO₂ and eN had no effect on the biomass of bacteria and fungi or the fungi:bacteria ratio in the rhizosphere of either cultivar (Fig. S1a; Table 1). At the ripening stage, effects of eCO₂ on microbial community structure dominated, with PC1 clearly separating the communities under aCO₂ and eCO₂ (PC1 = 73.2% and PC2 = 14.0% of

the variation; Fig. S3b). eCO₂ tended to increase the overall microbial biomass at the ripening stage, increasing fungal biomass by up to 32% ($p < 0.05$; Fig. 3; Table 1), resulting in a significant increase of the fungi:bacteria ratio (Fig. S2b).

3.3. Soil microfauna

Repeated measures ANOVA confirmed a significant CO₂ effect on soil microfauna across rice growth stages. At the heading stage, flagellates were significantly reduced at eCO₂ under aN by 61% and 44% for weak- and strong-responsive cultivars, respectively (Fig. 4a). eN induced an overall increase in flagellate abundance of beyond 35% at the ripening stage, but the response to eCO₂ depended on an interaction between cultivar and N (Fig. 4a and 4c; Table 1). At the ripening stage, flagellates under weak-responsive cultivar increased on average 1.6-fold from aN to eN, irrespective of CO₂ level; also flagellates under strong-responsive cultivars increased 1.5-fold at eN, but only with eCO₂ (Fig. 4a and 4c). The amoebae increased by 31% under eCO₂ at the heading stage, but at the ripening stage the opposite trend was found under eN (Table 1; Fig. 4b and 4d).

Impacts of eCO₂ on the soil nematode depended on N dose, cultivar and growth stage (Fig. 5 and S4; Table 1). At the heading stage, eN increased bacterivorous nematodes by 41% (Fig. 5a) and this effect was maintained at the ripening stage but only for the weak-responsive cultivar under eCO₂ (Table 1; Fig. 5e). At the ripening stage, fungivorous nematodes increased 2.4-fold with eCO₂ at aN under strong-responsive cultivars, and 2-fold at eN under weak-responsive cultivars compared to aCO₂ (Table 1; Fig. 5f). Also at the ripening stage, omnivorous-carnivorous nematodes reached significantly higher densities at eCO₂, particularly at eN (Table 1; Fig. 5h).

The abundance of herbivorous nematodes consistently increased under the strong-responsive cultivar at eCO₂, independent of rice growth stages ($F = 18.91$, $p < 0.01$, Table 1; Fig. 5c and 5g). At the heading stage under eCO₂, the numbers of herbivores almost doubled ($\times 1.8$ under aN) and tripled ($\times 2.9$ under eN) under strong-responsive rice with eCO₂, while the numbers of herbivorous nematodes decreased by 59% under weak-responsive rice at eN (Fig. 5c; Table 1). At the ripening stage herbivores increased by 47% when eCO₂ plants received fertilizer, but decreased by 32% under weak-responsive cultivars (Fig. 5g; Table 1). On average, herbivores under strong-responsive cultivars increased 1.6-fold under eCO₂ irrespective of N fertilization. Overall, this resulted in a 1.6-fold increase of root herbivores at eN compared to aN under eCO₂ conditions (Table 1).

3.4. Effects of eCO₂ and eN on the structure and function of micro-food webs in the rhizosphere of rice

At the heading stage, eCO₂ and eN significantly increased the availability of soil resources, in particular DOC and DON (Fig. 2 and S6). However, these belowground inputs did not affect bacterial and fungal biomass (Fig. 3 and S6), likely due to enhanced turnovers of bacterial and fungal biomass, as indicated by significant higher numbers of bacterivores (nematodes, 26%; amoebae, 14%) and in particular the omnivorous-carnivorous nematodes (44%) at the third trophic level (Fig. S6).

At the ripening stage, eCO₂ and eN led to increased root biomass and this significantly increased the abundance of herbivorous nematode (Fig. 6). The increased root biomass correlated with an enhanced biomass of bacteria (covariance coefficient = 0.39, $p < 0.05$), and in particular of fungi (covariance coefficient = 0.69, $p < 0.01$; Fig. 6). eCO₂ and eN had a

direct impact on soil resources such as DOC and Ext N, and increased the total microbial biomass. Increased bacterial and fungal biomass was positively associated with the increased abundance of bacterivorous and fungivorous nematodes, respectively (Fig. 6). Interestingly, amoebae were directly related to root biomass and statistically marginally associated with fungi (covariance coefficient = 0.33, $p = 0.07$; Fig. 6). eCO_2 thus directly influenced resource availability for herbivores (root biomass) and microbes (DOC, Ext N) and subsequently propagated mainly via the fungal energy channel into the microbial primary consumers and then further up in the trophic chain to secondary consumers (i.e., omnivorous-carnivorous nematodes). In total, the model explained 69% of the variance in omnivorous-carnivorous nematodes, while the remaining relationships between variables were not significant but improved the fit of the model (Fig. 6).

4. Discussion

4.1. Responses of bacteria- and fungi-based energy channels of rhizosphere micro-food webs to eCO_2 and eN

Our results showed that positive effect of eCO_2 and eN on soil micro-food webs were mainly caused by the altered availability of C and N in the plant rhizosphere, confirming bottom-up control of the rhizosphere micro-food webs. eCO_2 often stimulates C allocation belowground, leading to increased microbial biomass and/or microbial respiration (Zak et al., 2000; Hu et al., 2001; Luo et al., 2004). CO_2 -enhancement of belowground C allocation likely occurs through increasing root growth and root exudation (Matamala et al., 2003; Norby et al., 2004), and mycorrhizal fungi (Cheng et al., 2012), providing new resources for microbial growth and

subsequent grazers (Blankinship et al., 2011; Mueller et al., 2016). At the ripening stage, with the root data available, it became clear that the main driving force came from roots (Fig. 6), especially considering the significant interactive effects of CO₂ and cultivars on root traits (Fig. S5). In addition, the eCO₂ effect will depend on N availability because the relative availability of C and N can either drive the bacteria- or fungi-based food web (Bååth et al., 1981; Mikola & Setälä, 1999).

The results of the present study showed that the effects of eCO₂ and eN on soil food webs can occur through altering biomass and/or turnover rates of each trophic level (Table 1). SEM indicated a clear dominance of the bacterial energy channel at the heading stage when peak plant growth occurs (Fig. S6). The fact that the growth of flagellates (Fig. 4a) and bacterivorous nematodes (Fig. 5a) was strongly restricted by N availability with eCO₂ suggests that bacterial growth was limited by low N availability resulting from the increased C availability via root exudation (Hoeksema et al., 2000) and competition between microorganisms and plant roots for N (Hu et al., 2001; Reich et al., 2006). Abundance of bacterivores is a long-term indicator of bacterial production, and this may explain why several previous studies in grasslands or forests observed no significant CO₂ effects on bacterial biomass or abundance in spite of increasing microbial N limitation (Ebersberger et al., 2004; Chung et al., 2006; Sinsabaugh et al., 2003). The increased bacterivore numbers under eN further point towards a strong top-down control over bacterial biomass (i.e. the rhizosphere microbial loop; see Clarholm, 1985; Bonkowski, 2004). Under elevated CO₂ when the relative availability of C to N was high, nutrient excretion by bacterivores can alleviate resource limitation of the grazed microbes to such an extent that reproductive rates of bacteria

keep up with grazing rates, increasing microbial turnover rates without detectable effect on the microbial biomass (Alpei et al., 1996; Frey et al., 2001; Trap et al., 2016). Also, similar magnitudes of increase in flagellates and bacterivorous nematodes (Fig. 4 and 5) suggests that food quality (C:N ratio of substrate and the specific populations of bacteria) rather than quantity (e.g. increased bacterial biomass) stimulated bacterivores (Schmidt et al., 2000; Cesarz et al., 2015). In contrast, eCO₂-induced changes in amoebae were independent of nitrogen status at the heading stage, indicating different trophic relationships in comparison with flagellates (Fig. 4b; Geisen, 2016). Also, the SEM indicated that amoebae were positively associated with root biomass (Fig. 6). Together, these results suggest that amoebae were related to enhanced root exudation associated with root growth promotion under eCO₂ and eN (i.e. the modified microbial loop; Bonkowski and Clarholm, 2012).

The alteration of bacterivores, especially nematodes and amoebae at the heading stage explained a large proportion of the variance in omnivorous-carnivorous nematodes (44%) at the third trophic level (Fig. S6). These results accord with the conceptual framework of the microbial loop, indicating a strong top-down control of bacterial biomass by bacterivores (Bonkowski, 2004; Neher et al., 2004; Wolkovich, 2016), with bottom-up root control and significant transfer of bacteria-derived C and N to higher trophic levels at the heading stage. Consistent with this notion, a shift in relative dominance occurred at the ripening stage from the former bacteria-based energy channel to a fungal-based energy channel under eCO₂ (Fig. S2). Nevertheless, the fungal-bacterial ratios in the paddy rice system were smaller than those of grasslands (Hungate et al., 2000). The lower proportion of fungal biomass in paddy rice may belie their importance, as the abundance of fungivorous nematodes under eCO₂ was

twice that of aCO₂ (Fig. 5f). Amoebae showed a clear connection to the fungal channel ($p = 0.07$; Fig. 6), supporting evidence of strong trophic links between amoebae and fungi in soils (Chakraborty et al., 1985; Geisen et al., 2016). Also, previous studies found that eCO₂ increased saprotrophic fungi (Drigo et al., 2007) as well as arbuscular mycorrhizal fungi (Drigo et al., 2013), but our results indicate that the plant's energy shunt to either bacteria or fungi is dynamic and switches among growth stages (Dunfield & Germida, 2003; Mougel et al., 2006; Houlden et al., 2008).

4.2. Interaction between rice cultivars and eCO₂ and eN determined herbivore load

Most previous studies on eCO₂ and eN effects on soil food web interactions focused on the decomposer food web and only a few studies included herbivores or parasitic microbes (Ayres et al., 2008; Cesarz et al., 2015; Chen et al., 2015). Plant parasitic nematodes can cause significant yield losses cereal production systems, including rice cropping systems (Bridge et al., 2005), but until now they received limited attention (Liu et al., 2008b; Huang et al., 2015), most probably due to their hidden form of herbivory (Johnson et al., 2016).

Elevated CO₂ levels may affect root herbivores in different ways. On the one hand, the increased biomass and growth rate of rice roots under eCO₂, particularly for the strong-responsive cultivar (Yang et al., 2008; Zhu et al., 2013), will increase food supply to root herbivores. On the other hand, eCO₂ could adversely affect the herbivores via reduced food quality (i.e. wider C:N ratio) (Norby & Cotrufo, 1998; Reich et al., 2006), even when high N was supplied (Sinclair et al., 2000). Therefore, improving crop yield by the selection of cultivars positively responsive to elevated CO₂ levels (Brummer et al., 2011; Ziska et al.,

2012) might mitigate against the predicted effects of future climate change (Cramer et al., 2001; Olesen & Bindi, 2002). However, our data clearly show that the positive response of rice cultivars to elevated CO₂ might come at a cost of increased herbivore load.

The doubling of the abundance of herbivorous nematodes with eN under the weak-responsive cultivars at the heading stage (Fig. 5c), and their reduction to control levels under eCO₂ could be explained by this reduced food quality for herbivores. Accordingly, the weak-responsive rice with its reduced root biomass and lower food quality supported reduced levels of herbivorous nematodes under eCO₂. In contrast, the performance of the strong-responsive cultivar to eCO₂ is highly dependent on N fertilization (Zhu et al., 2015). This led to improved resource quality (e.g. increased root N content and reduced C:N ratio) and quantity (e.g. root biomass) for herbivores (Fig. S4). Thus, the strong-responsive cultivars face a trade-off between N-limitation and herbivore load, especially if N-limitation is counterbalanced by fertilization.

Our results clearly demonstrate that management strategies intended to mitigate negative climate change effects on crops can lead to conditions conducive to plant parasite outbreaks. Therefore adopting high-yielding crop cultivars adapted to climate stress without taking into account root resistance to herbivores may imperil future crop production, and other global change factors, such as warming, may even exacerbate this effect (DeLucia et al., 2012).

Breeding new crop cultivars with improved resource use efficiency to satisfy food demand, as well as controlling invasive weeds and pathogens, is one of the most promising practices for agronomists under the pressure of the ongoing climate change (Bender et al., 2016; Brummer et al., 2011; Hirel et al., 2007). However, our findings clearly show that

highly productive cultivars under eCO₂ may raise pest infestation rates, suggesting an unexpected trade-off that would generate long-term negative soil feedbacks. Cultivars with comprehensive traits should be taken into account in future integrated crop management (Huang et al., 2012; Tiemann et al., 2015).

5. Conclusions and outlook

Our results showed differential responses of soil microbes and microbivores to eCO₂ and N inputs at different growing stages of rice. These results illustrated the highly temporal-dynamic nature of soil micro-food web responses to the changing climate conditions and call for caution in extrapolating results from single sampling time and/or single trophic level to predict the long-term impact of climate change factors on the soil micro-food web. Also, the interactive effect of eCO₂, eN and cultivars on soil food web indicated that alteration in resource availability to microbes can cascade up along the food web. eCO₂ in general increases the C:N ratio of plant materials and thus reduces the quality for herbivores, which are often assumed to have negative effects on herbivorous nematodes and other pest insects. However, strong-responsive cultivar was susceptible to root-feeding pests under elevated CO₂ and N fertilization, thus rendering the long-term advantage of breeding positively CO₂-responding cultivars questionable. Regarding to agricultural managements under future climate change scenarios, this study highlights crop breeding strategies should integrate knowledge about the architecture and metabolic footprints of soil food web.

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673 **Table 1.** *F* value of repeated measures ANOVA on the effects of CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous
674 [eN]), Cultivar (weak- and strong-responsive) and all possible interactions on the biomass of microorganisms and the abundance of protists,
675 nematodes and nematode trophic groups across sampling times (T) at heading and ripening growth stage.

	Microbial biomass	Bacterial biomass	Fungal biomass	Flagellates	Amoebae	Total nematode	Bacterivores	Fungivores	Herbivores	Omnivores- carnivores
eCO ₂	1.47	0.63	5.02*	3.08	4.82*	4.98*	0.01	4.56*	1.96	10.89**
eN	2.21	1.75	3.65	35.93**	0.53	51.80**	19.79**	0.46	29.90**	20.89**
Cultivar	0.84	0.85	1.48	0.18	0.09	20.31**	0.16	0.10	24.56**	4.91*
eCO ₂ × eN	0.40	0.17	0.15	12.92**	0.05	6.41*	6.35*	0.65	1.27	7.97*
eCO ₂ × Cultivar	0.07	0.07	0.00	7.34*	0.43	29.14**	0.03	0.02	58.39**	1.94
eN × Cultivar	0.04	0.00	0.09	5.99*	0.01	9.88**	3.45	8.81**	5.19*	1.61
eCO ₂ × eN × Cultivar	0.01	0.12	0.47	5.23*	0.58	1.48	10.70**	4.91**	1.13	2.57
T	51.17**	50.32**	48.22**	8.46*	0.34	45.81**	42.24**	23.87**	4.99*	45.81**
T × eCO ₂	1.85	0.46	4.50*	5.01*	5.87*	17.10**	0.09	13.21**	11.38**	17.10**
T × eN	0.02	0.01	1.33	0.12	0.91	0.30	0.32	1.19	0.24	0.30
T × Cultivar	2.57	3.12	0.81	0.07	0.19	13.09**	0.24	3.78	6.45*	13.09**
T × eCO ₂ × eN	0.35	0.41	0.09	2.63	5.45*	9.32**	1.23	2.38	10.97**	9.32**
T × eCO ₂ × Cultivar	0.22	0.32	0.08	0.003	0.49	31.91**	7.17*	2.89	18.91**	31.91**
T × eN × Cultivar	0.76	0.67	0.04	0.16	1.65	0.2	1.61	1.76	2.13	0.20
T × eCO ₂ × eN × Cultivar	0.42	0.72	0.01	0.56	1.26	38.63**	9.58**	6.96*	19.70**	38.63**

676 *, ** indicates factors effect significant at $p < 0.05$, $p < 0.01$, respectively

Figure captions

Fig. 1 Photograph showing one of the three FACE rings in Zongcun Village (119°42'0" E, 32°35'5" N), Yangzhou City, Jiangsu Province, China.

Fig. 2 The content of soil resources in varying CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous [eN]), and Cultivar (weak- and strong-responsive) treatment (a: Dissolved organic carbon, n = 12; b: Dissolved organic nitrogen, n = 12 and 6 at the heading and ripening stage, respectively; c: Mineral nitrogen, n = 12). Only significantly different results are presented, and see Table S2 and S3 for all data of soil resources. Means with different letters indicate significant difference among treatments (Fisher's LSD test, $p < 0.05$). Error bars are standard errors.

Fig. 3 The biomass of the overall microbial community, bacteria and fungi in varying CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous [eN]), and Cultivar (weak- and strong-responsive) treatment (a-c: Heading stage; d-f: Ripening stage). Means with different letters indicate significant difference among treatments (Fisher's LSD test, $p < 0.05$). Error bars are standard errors (n = 3).

Fig. 4 The abundance of flagellates and amoebae in varying CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous [eN]), and Cultivar (weak- and strong-responsive) treatment (a, b: Heading stage; c, d: Ripening stage). Means with different letters indicate significant difference among treatments (Fisher's LSD test, $p < 0.05$). Error bars are standard errors (n = 3).

Fig. 5 The abundance of nematode trophic groups in varying CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous [eN]), and Cultivar (weak- and

strong-responsive) treatment (a-d: Heading stage; e-h: Ripening stage). Means with different letters indicate significant difference among treatments (Fisher's LSD test, $p < 0.05$). Error bars are standard errors ($n = 3$).

Fig. 6 Structural equation modeling (SEM) analysis of the elevated CO₂ and N fertilization effects on soil micro-food webs at the ripening stage in a rice field in Jiangsu province, China. The results of the optimal model fitting [Chi-square (χ^2) = 21.755, $df = 10$, $p = 0.061$, comparative fit index (CFI) = 0.937, root square mean error of approximation (RMSEA) = 0.221]. Square boxes denote variables include in the models. Values associated with solid and dashed arrows represent standardized path coefficients. Percentages close to variables indicate the proportion of variation explained by the model (R^2). Solid arrows denote the directions and effects that were significant ($p < 0.05$) and the thickness represents the magnitude of the path coefficients. Dashed arrows represent the directions and effects were non-significant ($p > 0.05$). (ExtN; extractable N, the sum of NH₄⁺-N, NO₃⁻-N and DON; BF: bacterivores; FF: fungivores; HE: herbivores; OC: omnivores-carnivores; Flag: flagellates; Amoe: Amoebae)



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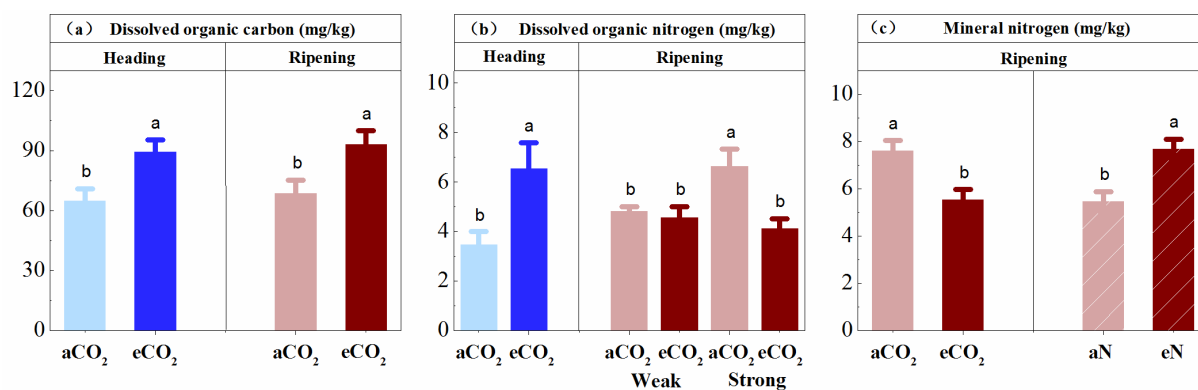


Fig. 2 The content of soil resources in varying CO₂ (ambient [aCO₂] and elevated [eCO₂]), N (ambient [aN] and exogenous [eN]), and Cultivar (weak- and strong-responsive) treatment (a: Dissolved organic carbon, n = 12; b: Dissolved organic nitrogen, n = 12 and 6 at the heading and ripening stage, respectively; c: Mineral nitrogen, n = 12). Only significantly different results are presented, for all data of soil resources, see Table S1 and S2. Means with different letters indicate significant difference among treatments (Fisher's LSD test, $p < 0.05$). Error bars are standard errors.

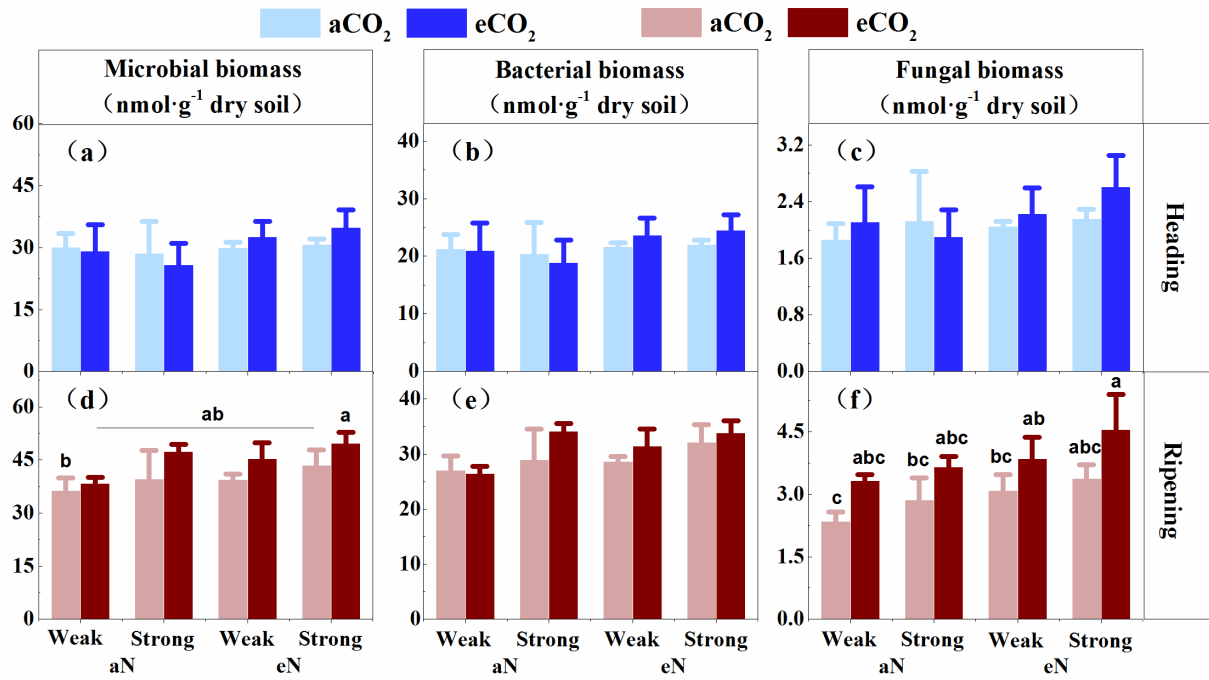


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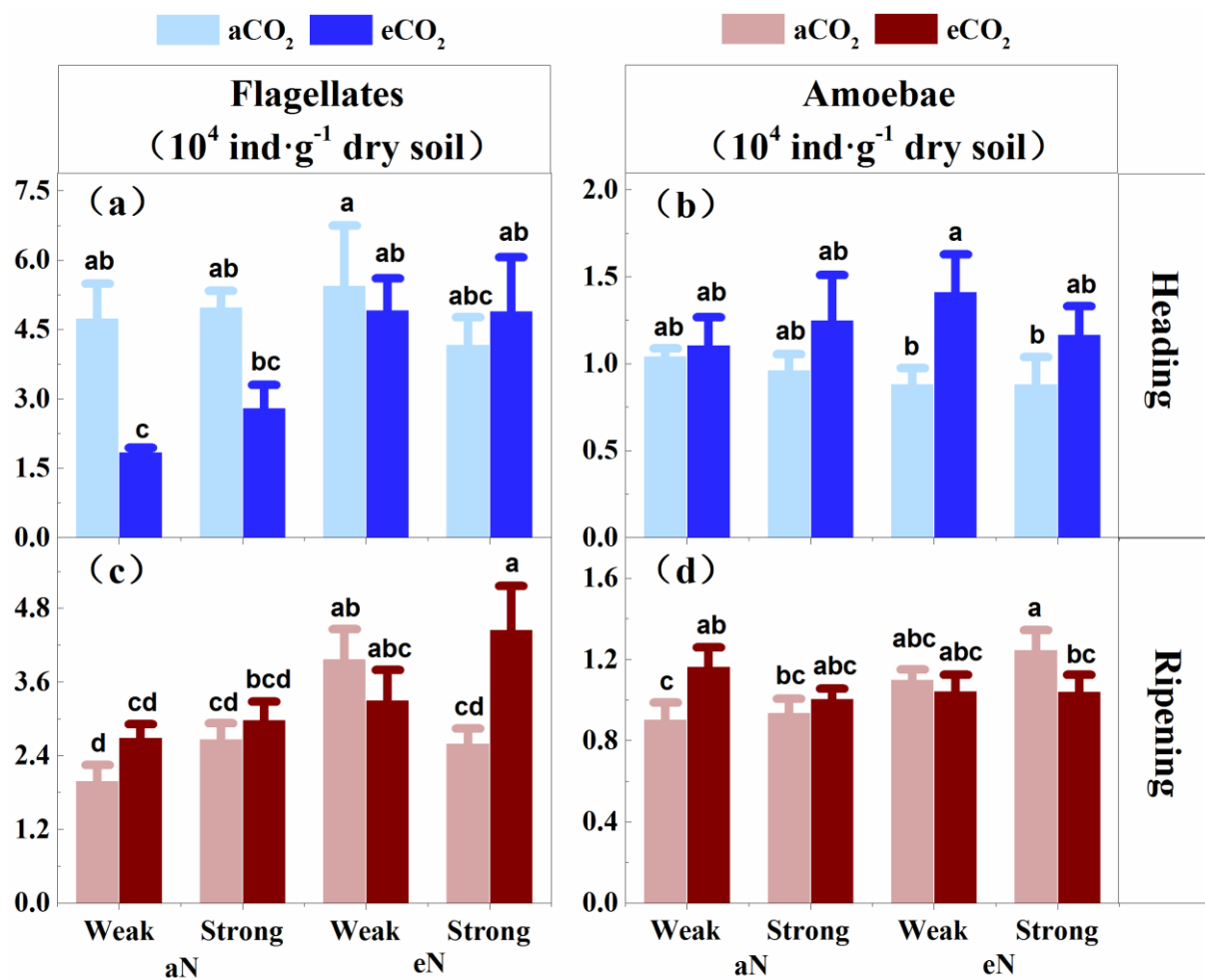


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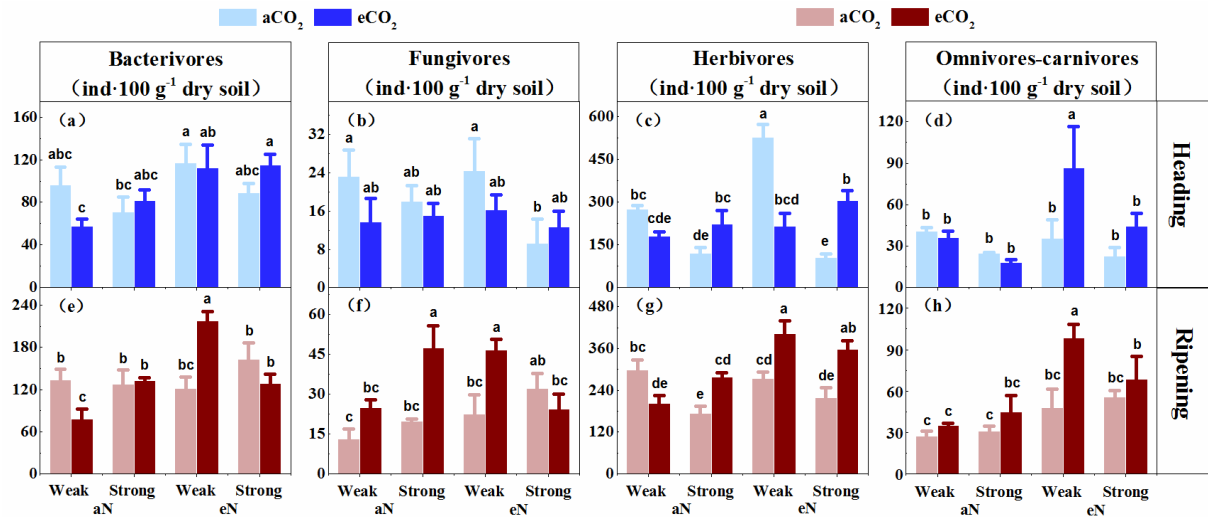


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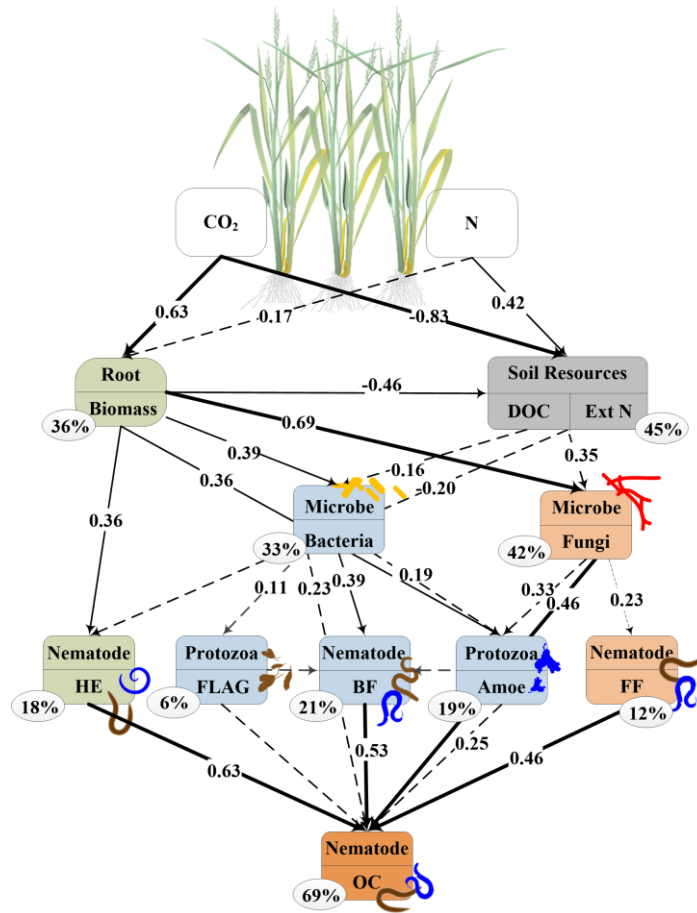


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